

Abstract

Two biological control agents of *Serratia plymuthica*, strains IC1270 and IC14 applied separately and in combination were evaluated for suppressing *Penicillium digitatum* (green mould) or *Penicillium italicum* (blue mould) on orange. These bacteria were effective and controlled both pathogens at 1×10^8 cells/mL. Disease suppression was increased when both bacterial strains were combined. Possible modes of action studied in this work were antibiosis, chitinolytic activity and competition for nutrients. Two mutants of strain IC1270, one deficient in chitinolytic activity and the second deficient in pyrrolnitrin production were obtained by the gene replacement technique. On orange fruit, mutant IC1270-C7 deficient in chitinase production and mutant IC1270-P1 deficient in pyrrolnitrin production showed a similar efficiency against *P. digitatum* to the parental strain. However, in vitro IC1270-P1 lost its antifungal activity and no inhibition zone was observed when it was tested against *P. digitatum* or *P. italicum*. In similar experiments, a chitinase-deficient mutant of strain IC14 was as effective as the parental strain IC14, suggesting no evidence for a possible role of chitinases in controlling green mould caused by *P. digitatum*. Interactions between strains IC1270 or IC14 and *P. digitatum* were studied in tissue culture plates with diluted orange peel extract as the nutrient source. Strain IC1270 decreased germination of *P. digitatum* conidia when it was physically separated from the pathogen by a membrane filter, which permits nutrient and metabolite interchange, while strain IC14 did not affect germination. Significant inhibition of conidial germination of *P. digitatum* was achieved, however, when the pathogen and IC14 were in physical contact. Competition for nutrients appears to be the main mode of action of strain IC1270, while a direct cell-to-cell interaction between IC14 and the pathogen is needed for antagonism.